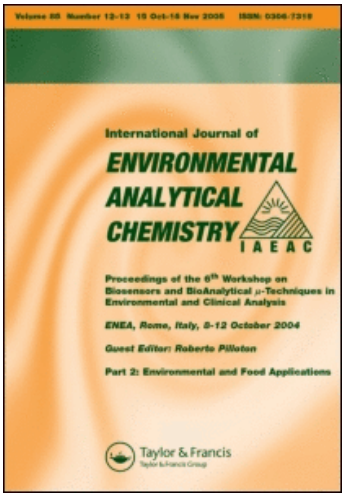

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Continuous Flow Determination of Organophosphorus Pesticides Using Solid Phase Extraction Coupled On-Line with High Performance Liquid Chromatography

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CONTINUOUS FLOW DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES USING SOLID PHASE EXTRACTION COUPLED ON-LINE WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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A XAD-2 resin is used for trace enrichment of three organophosphorus pesticides (diazinon, azinphos-methyl and fenthion) on an unsegmented-flow solid-phase preconcentration system, coupled on-line with a high performance liquid chromatograph with UV detection at 220 nm. The influence of different parameters (e.g. pesticides water concentrations, water pH, precolumn characteristics, preconcentration and elution times and solvent flow rates) on the retention and recovery of the pesticides was studied. Recoveries higher than 95%, were obtained for the three pesticides, except for diazinon at sample volumes higher than 500 ml. Calibration graphs are linear in the range of 0.07–2 mg l^{-1} for each pesticide under standard conditions, although concentrations down to 5 $\mu g\, l^{-1}$ can be measured. The method has been successfully applied to spiked river water samples.

KEY WORDS: Organophosphorus pesticides, solid phase extraction, HPLC-UV, residue analysis.

INTRODUCTION

An increasing number and amount of organophosphorus pesticides, which have been mainly developed to replace the more persistent organochlorine derivatives, are used in agriculture. Unfortunately, a certain percentage of these compounds reaches the aquatic environment in spite of their relatively rapid degradation rate and low bioaccumulation potential[1,2].

Consequently, to comply with the growing demand of residue data of such pesticides, more rapid and cost-effective analytical procedures are required. To date, there is no universal on-line technique for monitoring these compounds in water, but continuous-flow methodologies at low pressure[3,4] may offer great possibilities with segmented[5] and unsegmented[6] systems, provided that the problems of preconcentration and clean-up of the analyte traces in natural samples are solved. The most common separation methods for the treatment of water samples are liquid-liquid

extraction and solid phase extraction[7,8]. As regards to analytical methodologies, organophosphorus pesticides are currently determined by capillary gas chromatography (GC) with thermionic[9,10] or flame photometric detection[11]. However, when on-line preconcentration and clean-up of water samples, using liquid-liquid or solid-liquid extraction is carried out, the final extract can contain some water that will damage the GC column. In that case, the determination of pesticides exhibiting a chromophore moiety should be performed by means of high performance liquid chromatography (HPLC) using UV detection[12].

In a previous work[13], a continuous flow extraction system was coupled on-line with HPLC for the determination of some organophosphorus pesticides, using *n*-heptane as organic phase. However, some problems appeared due to the difficulties of obtaining high preconcentration factors. Alternatively, it has been shown that the use of adsorption techniques can overcome these problems in the determination of a great number of organic compounds[14–16], including organophosphorus pesticides. Polymeric resins[17–22] and C_{18} bonded phases[23] have been commonly used for solid-phase extraction for trace-enrichment of aqueous pesticide samples.

Some authors[24,25,26] have used adsorption resin columns with a high pressure preconcentration pump system coupled to a HPLC, but a low pressure pump system has not been applied to the determination of low concentrations of organophosphorus pesticides in natural waters.

In this work, water samples containing trace levels of organophosphorus pesticides have been analyzed by a low pressure unsegmented completely continuous flow system (CCFA) using Amberlite XAD-2 resin coupled on-line with a HPLC-UV detector. Three organophosphorus pesticides, commonly used in Spain: diazinon, azinphos-methyl and fenthion, have been selected for this study.

EXPERIMENTAL

Reagents

Organophosphorus pesticides (azinphos-methyl, diazinon and fenthion) were supplied by Riedel-de-Häen (Seelze, FRG) and HPLC-grade organic solvents by Scharlau (Barcelona, Spain). Distilled water was microfiltered through Millipore filters (0.45 μm) (Bedford, MA, USA). Standard aqueous solutions were prepared from dilutions of methanolic stock solutions.

Amberlite XAD-2 (poly-*p*-divinylbenzene-styrene) resin (Merck, Darmstadt, FRG) with particle size between 0.3–0.9 mm was used after purification by sequential Soxhlet extraction with methanol and acetonitrile and storage under methanol, according to the method of Junk *et al*[14]. Low blanks were obtained under these conditions.

Instrumentation

The HPLC system consisted of a Spectra-Physics (San José, CA, USA) Model SP-8700 solvent delivery unit, an injection valve Rheodyne (Cotati, CA, USA) with

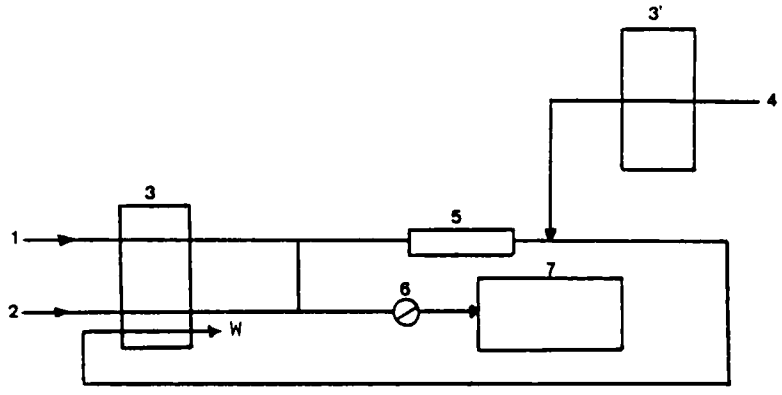

Figure 1 Schematic diagram of the manifold. 1 water sample, 2 pure water, 3 and 3'peristaltic pumps, 4 eluent of the preconcentration resin column, 5 resin column, 6 HPLC injection valve, 7 HPLC-UV detector, w waste.

a 10 μl sample loop, an UV-Vis detector (SP-8440), an integrator (SP-4270) and a Brownlee Labs RP-18 column (220 × 4 mm i.d.). The working wavelength was 220 nm. The mobile phase was 80:20 v/v methanol/water and its flow rate was set at 1 ml min^{-1}. Quantitative analyses were based on peak heights.

Under these conditions, the retention times of the pesticides were: diazinon (1.5 min), azinphos-methyl (3.7 min) and fenthion (4.7 min). No differences in these values were observed when samples in water and methanol were injected.

Continuous flow manifold

The continuous flow system consisted of two Watson-Marlow 202 U/AA4 (Cornwall, UK) peristaltic pumps and home-made glass columns. Standard Tygon pump tubing and Teflon tubing (0.5 mm i.d.) were used in the system.

Figure 1 shows the flow manifold. The water sample (1) is introduced by the peristaltic pump (3) and the pesticides are quantitatively retained in the XAD resin (5). Immediately after, by stopping pump (3) and starting pump (3'), the pesticides are eluted by the organic eluent, fed into the HPLC injection valve and finally introduced into the HPLC column.

RESULTS AND DISCUSSION

Retention and recovery of the organophosphorus pesticides

Among the various types of XAD solid phase adsorbents, XAD-2 was selected for this study. The column size was 50 mm × 3 mm i.d. and the packing density was 0.25 g cm^{-3}.

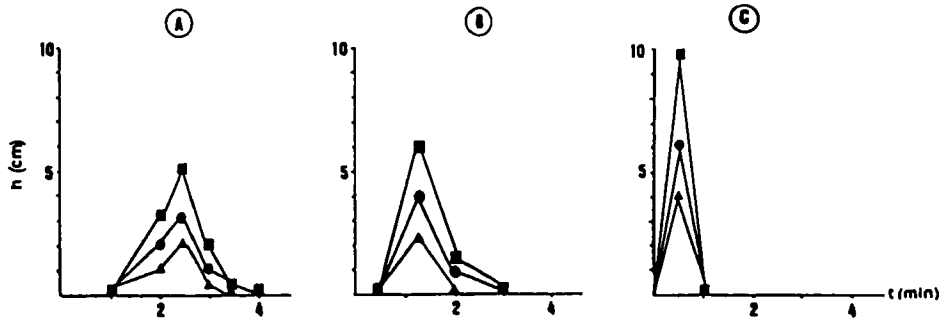

Figure 2 Elution profile from the preconcentration column (XAD-2 resin) at three eluent flow rates. A) 0.7 ml min^{-1}, B) 1.6 ml min^{-1}, C) 2.5 ml min^{-1}. Initial concentration 1 mg l^{-1} of each pesticide. Aqueous flow rate 3.8 ml min^{-1}. Preconcentration time 10 min. ● Diazinon; ■ Azinphos-methyl; ▲ Fenthion.

The retention of diazinon, azinphos-methyl and fenthion in aqueous samples was quantitative in the concentration range of 0.5 to 10 mg l^{-1} and irrespectively of the preconcentration aqueous flow rate, in the range of 1 to 6 ml min^{-1}. Apparently, no saturation of the resin was observed after the adsorption of 10 mg of each pesticide per gram of resin. The elution of these compounds was performed with methanol and *n*-heptane, since both solvents do not absorb at the working wavelength (220 nm). The results showed a recovery of 95% of the pesticides with 5 ml of methanol and of only 5% with 5 ml of *n*-heptane. Therefore, methanol was the selected eluent for further work. The resin can be continuously used during a whole day and it can be regenerated without losses of efficiency by using the Junk's treatment mentioned above.

During the elution of the pesticides, there is a maximum on the profile from the preconcentration column (see Figure 2). On-line analysis requires that the injection of the sample into the chromatographic column match this maximum. Therefore, to obtain the highest sensitivity and a good reproducibility the elution time has to be accurately controlled. Changes in the shape of the chromatographic signal with methanol flow rate and initial pesticide concentration are shown in Figures 2 and 3.

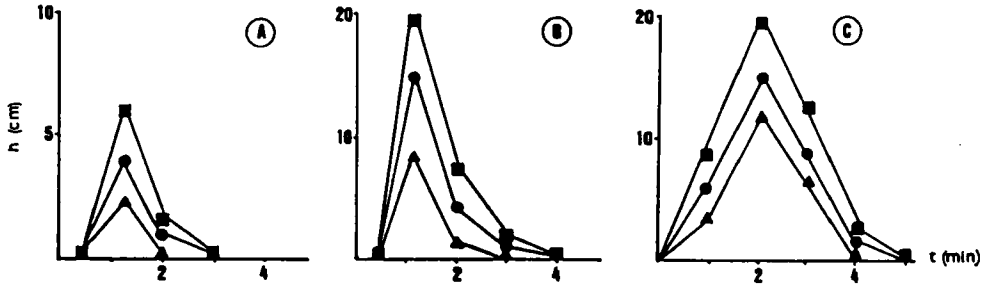

Figure 3 Elution profile from preconcentration column at three initial pesticide concentrations. A) 1 mg l^{-1}, B) 4 mg l^{-1}, C) 10 mg l^{-1}. Eluent flow rate 1.6 ml min^{-1}. Other conditions as in Figure 2.

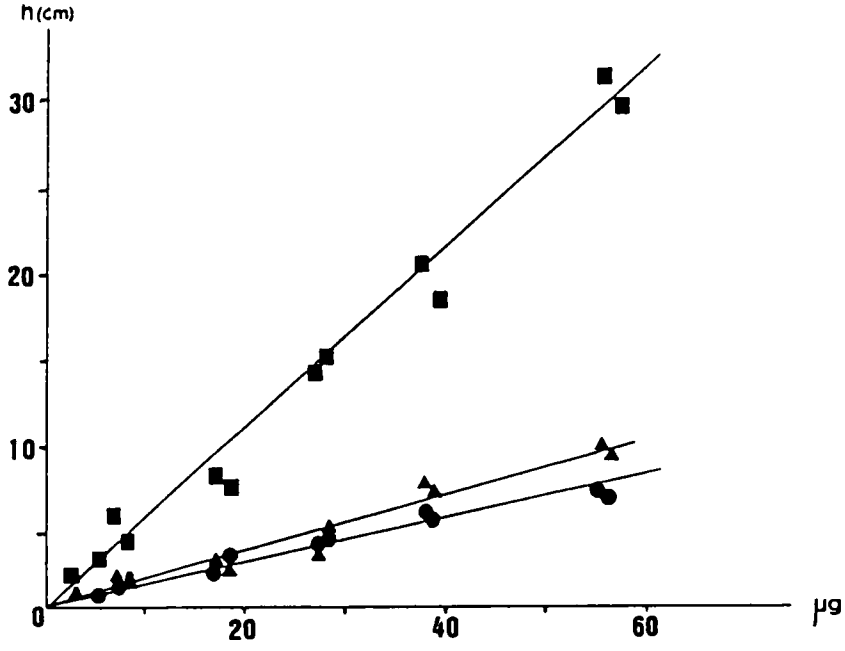

Figure 4 Chromatographic signal vs the amount of the pesticides retained at different preconcentration times, sample flow rates and initial pesticide concentrations. Eluent flow rate, 1.6 ml min^{-1}. ● Diazinon; ■ Azinphos-methyl; ▲ Fenthion.

A flow rate of 1.6 ml min^{-1} was chosen because it provided a reproducible signal in a relatively short elution time (70 s). If the initial concentrations of pesticides are lower than 5 mg l^{-1}, the elution time corresponding to their maximum chromatographic signals is practically the same. According to these results the conditions used in the following experiments were: methanol flow rate 1.6 ml min^{-1} and pesticide concentrations up to 5 mg l^{-1}.

Table 1 Recovery of the three pesticides at different initial concentration and preconcentration time. Aqueous flow rate: 3.6 ml min^{-1}. (D = diazinon, Az-Me = azinphos-methyl and F = fenthion).

Initial concentration (*mg* l^{-1})	*Preconcentration time* (*min*)	*Recovery* (%)		
		D	*Az-Me*	*F*
0.01	570	10	>95	>95
0.02	250	50	>95	>95
0.07	10	>95	>95	>95

Study of the continuous system

A set of experiments were carried out to study the influence of different parameters on the preconcentration of aqueous pesticide samples.

Preconcentration column characteristics. Two home-made glass precolumns with lengths of 50 and 180 mm and 3 mm I.D. were packed with 0.16 and 0.3 g of XAD-2, respectively. Experiments were carried out at initial pesticide concentrations of 0.25 and 0.50 mg l^{-1}, sample flow rate 3.8 ml min^{-1} and preconcentration time 10 min. Results indicated no differences in retention and recovery of the three pesticides and that elution time was independent of which column was used. Hence, a 50 mm length column and an elution time of 70 s were selected for further experiments.

Sample preconcentration. In order to see that the chromatographic signal only depends on the amount of pesticides retained in the preconcentration column, a set of experiments were carried out changing the initial pesticide concentration, sample flow rate and preconcentration time, representing a variation of the mass retained in the column from 3 to 57 μg. Figure 4 shows a good linear correlation between the chromatographic peak height and the retained mass of each pesticide.

Some other experiments were carried out at lower concentrations (0.01–0.07 mg l^{-1}). These results are shown in Table 1, where it can be observed that the preconcentration time does not affect the high recovery (>95%) of azinphos-methyl and fenthion, although diazinon exhibits a poor result. Taking into account that the degradation rate of diazinon is low[1,27] at the conditions of this study, the breakthrough of diazinon is probably the responsible, due to its higher polarity. This drawback can be minimized using lower sample volumes or increasing the precolumn length[24].

pH. All the experiments, mentioned above, were carried out at pH = 6. Some experiments were performed at pH = 4 and pH = 8 (initial concentration 1 mg l^{-1}, preconcentration time 10 min, elution flow rate 1.6 ml min^{-1} and elution time 70 s). No differences were observed on retention and recovery at these values. Therefore, this method can be applied to natural waters without modifying the pH if it is in the range mentioned above.

Characteristics of the flow method

Linearity range. Aqueous standard solutions of the pesticides at concentrations from 0.07 to 2 mg l^{-1} were pumped at a constant flow rate of 3.8 ml min^{-1} and a preconcentration time of 10 min. The elution time was 70 s and the methanol flow rate 1.6 ml min^{-1}.

The three pesticides provided good linearity in the concentration range studied. Equations of the straight lines obtained by linear regression were: $h = -0.08 + 3.78C$ ($r = 0.995$) for diazinon, $h = 0.39 + 21.0C$ ($r = 0.998$) for azinphos-methyl and $h = -0.37 + 8.2C$ ($r = 0.996$) for fenthion, where h is the chromatographic peak height in cm and C the initial concentration in mg l^{-1}.

Detection limit. The instrumental detection limit is 90 $\mu g\,l^{-1}$ for diazinon and fenthion and 40 $\mu g\,l^{-1}$ for azinphos-methyl, using a sample loop of 10 μl, at a signal-to-noise ratio of 3. In this way, water samples with concentrations down to 5 $\mu g\,l^{-1}$ of each pesticide can be analyzed with the preconcentration methodology described previously.

Precision. In order to evaluate the relative standard deviation (RSD) of the method, a set of sixteen solutions containing 0.3 $mg\,l^{-1}$ of each pesticide was analyzed. The RSD values were 10%, 12% and 11% for diazinon, azinphos-methyl and fenthion, respectively.

Sample throughput. The sample rate mainly depends on the preconcentration time. At the working conditions of this study, 6 samples per hour can be analyzed.

Interferences. The analysis of these pesticides, each one at 0.5 $mg\,l^{-1}$, was carried out in the presence of some other pesticides and related compounds. These compounds and their concentrations were: triclorforn (0.6 $mg\,l^{-1}$), DDE (0.4 $mg\,l^{-1}$), phenol (1.1 $mg\,l^{-1}$) *p*-nitrophenol (0.2 $mg\,l^{-1}$) and parathion-methyl (0.4 $mg\,l^{-1}$). The experimental conditions were the same as those used to obtain the calibration curves. The results showed that using this CCFA methodology, fenthion can be well determined, while phenols mask the diazinon peak and parathion-methyl coelutes with azinphos-methyl. These problems were overcome using as chromatographic mobile phase methanol:water (70:30)[27].

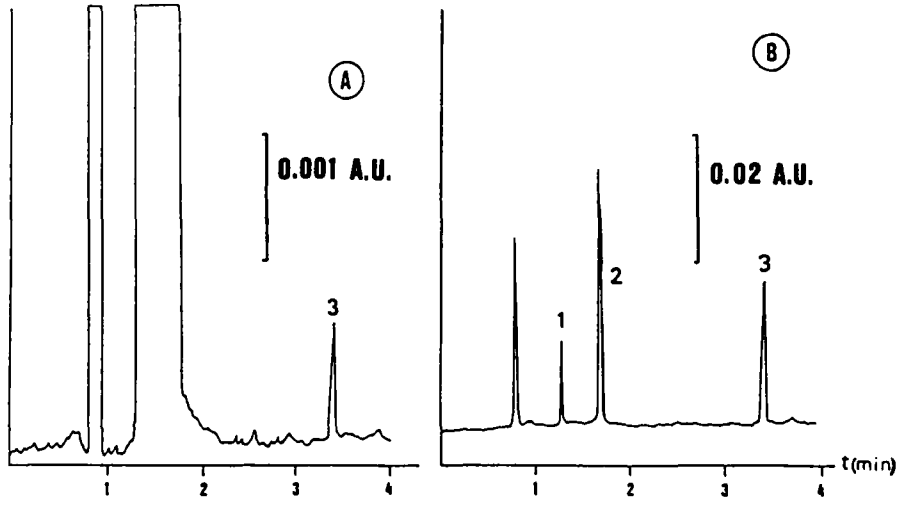

Figure 5 Chromatograms corresponding to spiked river water sample containing 0.5 $mg\,l^{-1}$ of each pesticide. (1) Diazinon, (2) Azinphos-methyl, (3) Fenthion. A) direct injection into the HPLC system, B) using the preconcentration flow system.

Application to pesticide residue analysis

This method has been applied to the determination of diazinon, azinphos-methyl and fenthion in water samples from a river located near Barcelona (Llobregat), spiked with 0.5 mg l^{-1} of each pesticide. Figure 5 shows the chromatograms from these experiments: A) direct injection of the river water into the chromatograph and B) the same sample using the enrichment methodology described in this work. Chromatogram 5A shows that only the determination of fenthion is possible, because the interferences mask the diazinon and azinphos-methyl peaks. On the contrary, the same sample passed through the flow system (preconcentration ratio = 15) gives the chromatogram of Figure 5B, where the three pesticides are well determined with recoveries higher than 90% in all cases.

CONCLUSIONS

The proposed method allows to determine traces of organophosphorus pesticides from a low-cost and low-pressure home-made glass preconcentration column system, coupled on-line to a high performance liquid chromatograph. At the same time, this solid phase precolumn enrichment system, combined with an unsegmented flow system, provides an easy, fast and non-contaminating sample handling setup. These characteristics, together with the satisfactory analytical performance of the method make it unsuitable for monitoring organophosphorus pesticides in natural and wastewater samples.

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